Supplementary Materials

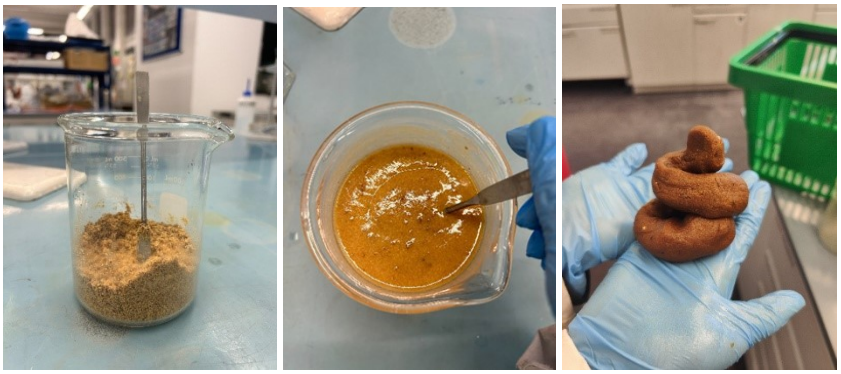
1. Methodology

1.1 Simulation of synthetic human faeces and urine

The selected recipes of human faeces and urine were originally developed by [1] and [2], respectively, and then were modified by [3,4]. Table S1 shows the recipe used in this study to simulate human faeces and urine. Yeast extract, cellulose, Psyllium husk, NaCl, KCl, and $CaCl_2$ were added first and mixed, followed by adding oleic acid and miso pastes, and these were mixed thoroughly before adding water to adjust TS content needed (Photo S1a). Miso pastes and psyllium which were purchased from WholeFoods as Clearspring Organic Japanese Miso Soup Paste and KIKI Organic Psyllium Husk. Slow stirring is needed when adding water until the mixture is gelled fully (Photo S1b) and then the synthetic faeces are ready to use. Distilled water can be added in any amount as required and 80% of the total wet weight was used in this study (Table S1). Fig.1c presents the different rheology of the synthetic faeces when adding ~30 %wwt distilled water, as an example. The synthetic human excreta are then refrigerated at 4 °C until being used. To simulate human excreta, the ratio of faeces and urine simulants is in accord with their reported daily production– 120 g wet faeces simulants to 300 mL urine simulants [3].

Table S1 Recipes used in this study to simulate human excreta [3,4].

Synthetic faeces		**Synthetic urine**	
Component	Composition in dry weight (dwt%)	Component	Amount (g/L)
Yeast extract	30	Urea	14.2
Microcrystalline cellulose	10	Creatinine	3
Psyllium husk	17.5	Diammonium citrate	2
Miso pastes	17.5	NaCl	8
Oleic acid	20	KCl	1.65
NaCl	2	$KHSO_4$	0.5
KCl	2	$MgSO_4$	0.2
$CaCl_2$	1	KH_2PO_4	1.75
Water	20 % of wet weight (wwt%)	$KHCO_3$	0.5



(a) (b) (c)

Photo S1. Simulation process of synthetic human faeces in the ratio of 20% dry weight and 80% distilled water

*Fi*FiFi

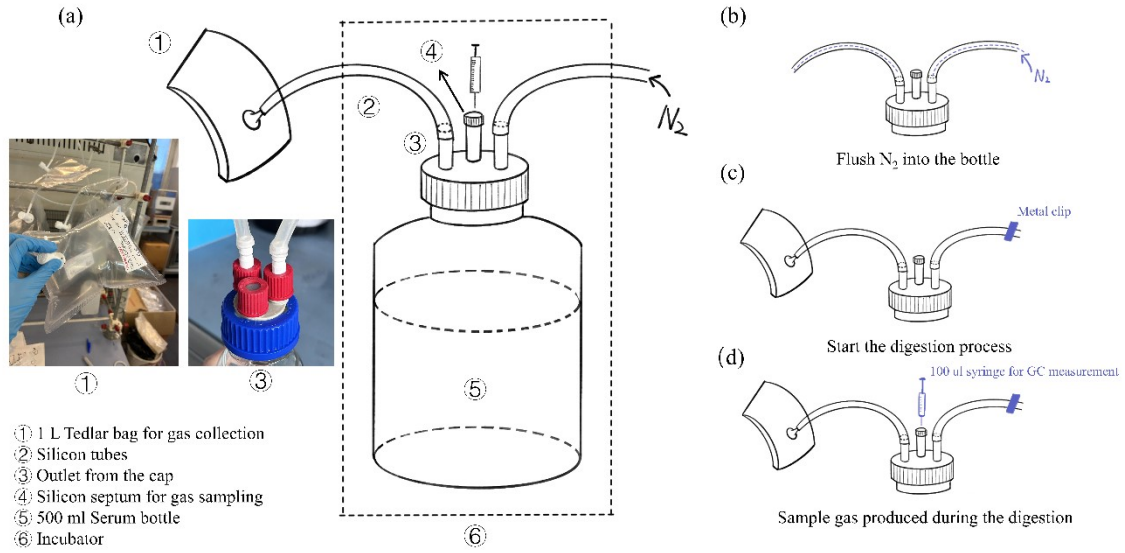


Figure S1. (a) Experiment set-up scheme and (b) working procedure for AD.

1.2 Supplementary characteristics of materials used in this study

Table S2. Characteristics of FSB from proximate and ultimate analysis.

Proximate analysis	**Volatile matter**	**Fixed carbon**	**Ash**	**Moisture content**
	19.95±2.36 %	8.43±2.20 %	66.54±0.98 %	5.08±0.72 %
Ultimate analysis	**C**	**H**	**N**	**S**
□	17.28±0.21 %	1.29±0.05 %	2.65±0.30 %	0.31±0.18 %

Table S3. Total concentrations of elements detected in simulated faeces and urine, inoculum, feedstock and biochar used in this study.

Metal	**Simulated faeces** (mg/L)	**Simulated urine** (mg/L)	**Inoculum** (mg/kg wwt*)	**Feedstock mixture** (mg/L)	**Biochar** (mg/kg TS)
Al	<LOD**	<LOD	4876.1 ± 20.5	730.8 ± 20.8	203181 ± 1667.6
Fe	<LOD	<LOD	16269.0 ± 187.0	2383.3 ± 370.8	28915.8 ± 1548.7
Zn	<LOD	<LOD	130.5 ± 3.8	<LOD	1402.4 ± 127.4
Cd	<LOD	<LOD	<LOD	<LOD	6.6 ± 1.3
Pb	<LOD	<LOD	<LOD	<LOD	89.0 ± 22.0
Cu	<LOD	<LOD	166.6 ± 0.3	<LOD	147.7 ± 9.4
Mg	85.2 ± 5.4	9.3 ± 1.6	781.1 ± 4.4	95.4 ± 3.8	28482.9 ± 2581.6
Li	<LOD	<LOD	<LOD	<LOD	203.4 ± 40.3
Ca	757.8 ± 22.8	<LOD	12798.0 ± 44.1	1709.2 ± 61.8	71015.8 ± 8327.8
Mn	<LOD	<LOD	<LOD	<LOD	1528.9 ± 117.0
Na	4241.0 ± 52.7	3498.0 ± 41.3	446.5 ± 33.0	731.8 ± 21.9	3101.5 ± 506.2

Co	<LOD	<LOD	<LOD	<LOD	5.6 ± 0.8
Cr	<LOD	<LOD	<LOD	<LOD	47.5 ± 2.8
Ni	<LOD	<LOD	<LOD	<LOD	28.3 ± 2.4
Ba	<LOD	<LOD	75.8 ± 0.1	<LOD	365.3 ± 25.6

Data are expressed as mean values (n=3) ± standard deviation.
**wwt: wet weight*
***LOD: Limit of Detection.*

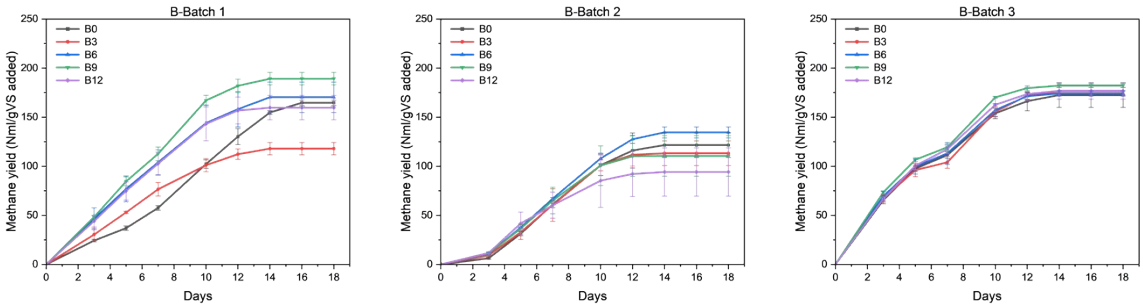
Table S4. Initial TS content after FSB addition.

Initial TS content	Average	STD
B0	4.38%	0.043%
B3	4.58%	0.042%
B6	4.78%	0.040%
B9	4.99%	0.039%
B12	5.19%	0.037%

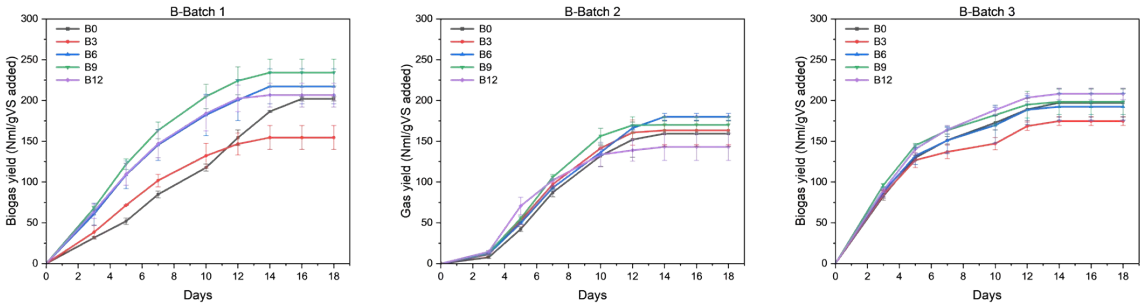
1.3 TN digestion method

Briefly, homogenised samples (0.400 ± 0.001 g) were weighed into 75 mL digestion tubes, including reagent blanks and quality control standards in each batch. A salicylic acid–sulfuric acid digestion mixture containing a selenium catalyst was added to each tube to retain nitrate/nitrite and catalyse the digestion. Samples were allowed to react for at least 2 h (or overnight), followed by heating at 100 °C for 2 h. After cooling, 30% (w/w) hydrogen peroxide was added dropwise to complete oxidation of organic matter. The samples were then further heated at 330 °C until digestion was complete and the digest became clear to pale yellow. After cooling, digests were diluted to 75.0 mL with distilled water and analysed for TN as ammonium using the Skalar San++ analyser.

2. Batch cumulative methane and gas yield



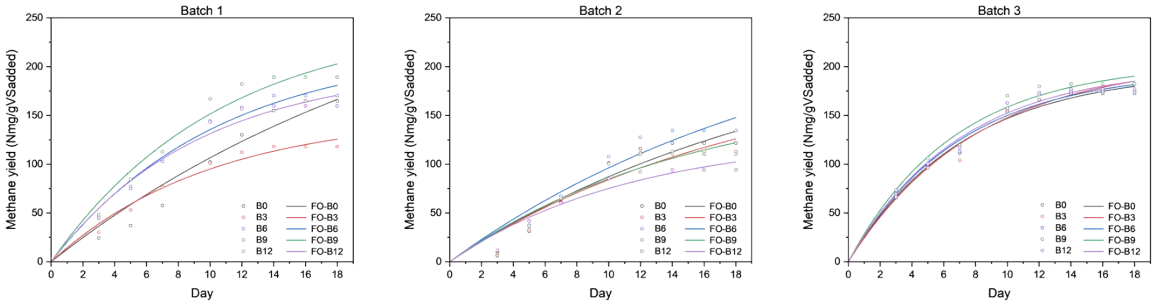
(a)



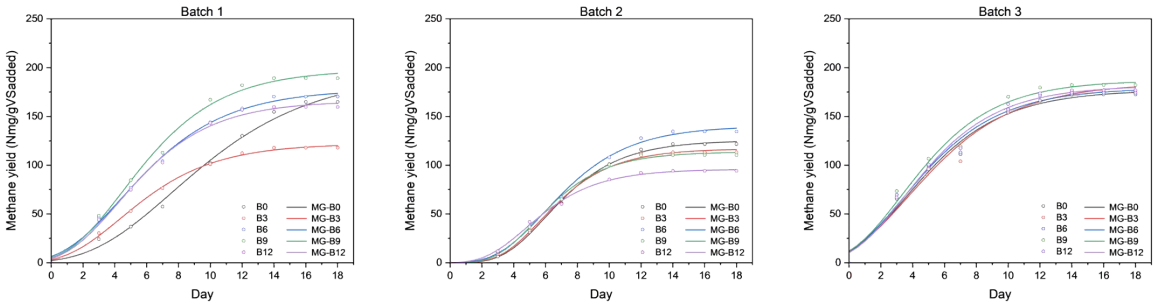
(b)

Figure S2. Cumulative (a) CH_4 and (b) gas yield from 3 batches of AD of synthetic human excreta with different biochar addition doses (n=3).

3. Kinetic models fitting



(a)



(b)

Figure S3. (a) First-order model and (b) Modified Gompertz model fitting to methane production from 3 batches of AD of synthetic human excreta with different biochar addition (N=3).

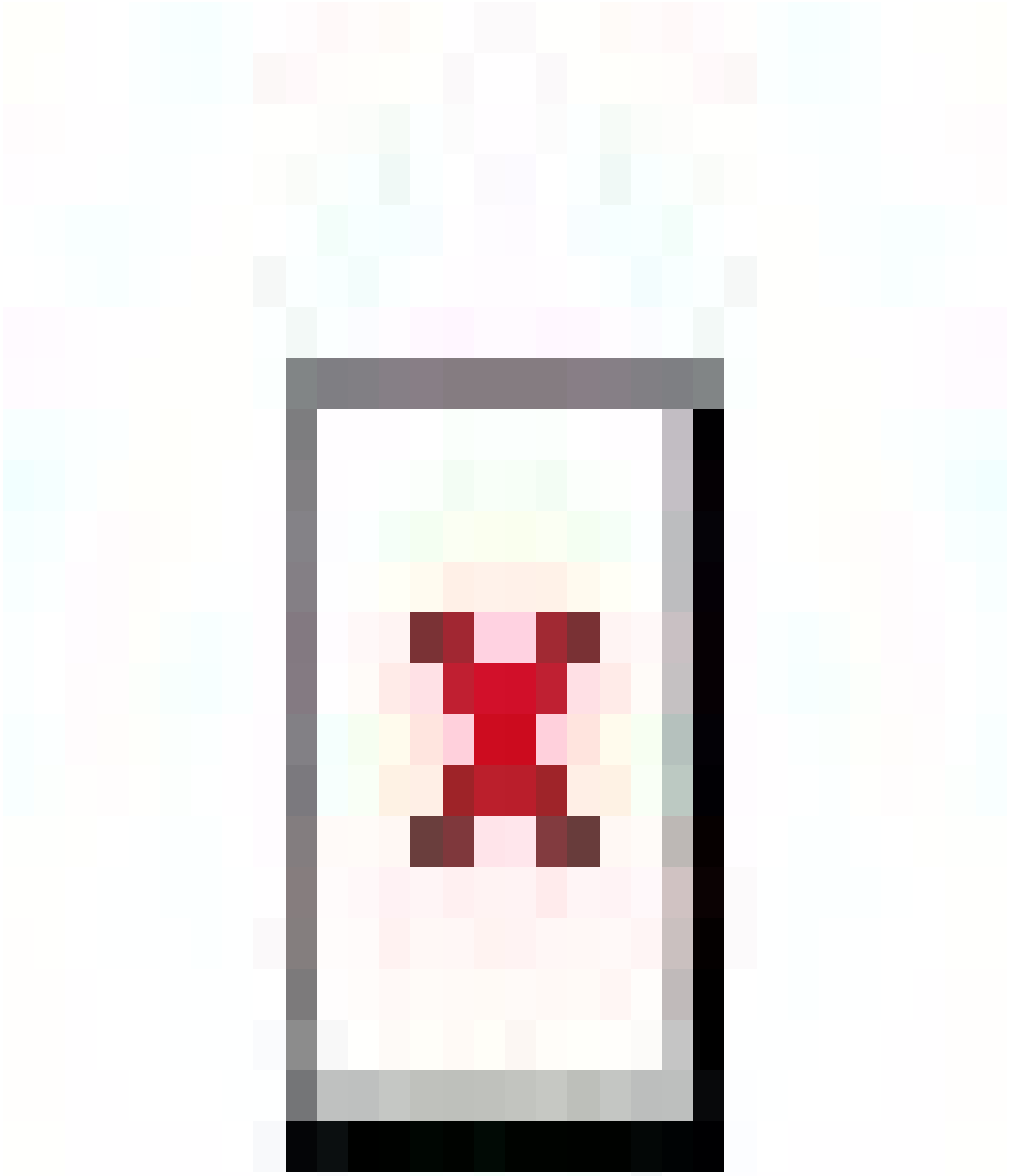


Figure S3. Digestate properties for each of the AD batch. Missing data was due to missing samples. * and **stands for significance level <0.05 and 0.01. VFA concentration is expressed as mg/l as Acetic acid.

Table S5. Heavy metal concentrations in Batch 1 AD feedstock mixture and digestate compared to limit for applying to agriculture and IC_{50} for AD reactions.

Batch 1	**□mg/L**	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
B0	□	<LOD	<LOD	<LOD	231.67±327.63	<LOD	22.52±30.96
B3	□	<LOD	<LOD	<LOD	29.58±41.84	<LOD	<LOD
B6	□	<LOD	<LOD	<LOD	198.33±253.58	<LOD	<LOD
B9	□	<LOD	<LOD	<LOD	7.50±7.50	<LOD	10.63±10.63
B12	□	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Feedstock mixture	□	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Limit on field [5]	South Africa	15.7	1750	50.5	200	N/A	N/A
	EU	20	1000	1000	300	N/A	N/A
IC_{50} [6,7]	Acidogenesis	29	17	0.9	440	880	3.5
	Methanogenesis	7.7	14.7	12.5	400	67.2	16

3.2 Microbial results

Table S6. Alpha diversity indices.

Sample_Name	chao1	goods_coverage	shannon	simpson
B12.batch 1a*	1126.952	0.999	5.964	0.935
B12.batch 1b	1122.714	0.999	5.936	0.931
B12.batch 2c	923.824	0.999	5.32	0.911
B12.batch 2a	904.549	1	5.214	0.882
B12.batch 2b	884.159	1	5.276	0.897
B12.batch 2c	894.556	0.999	4.694	0.821
B0.batch 1a	1097.78	1	6.14	0.955
B0.batch 1b	1067.819	0.999	6.124	0.956
B0.batch 2c	1129.801	1	6.239	0.955
B0.batch 2a	864.165	1	4.949	0.858
B0.batch 2b	983.808	0.999	5.444	0.906
B0.batch 2c	806.559	1	5.119	0.882

*a, b, and c represent triplicated samples.

Table S7. Relative abundance (%) of top30 genus annotated with AD related functions.

□	Taxonomy	B0_batch 1	B12_batch 1	B0_batch 2	B12_batch 2
Hydrolysis-Acidogenesis	Coprothermobacter	10.6651	14.5496	8.3028	7.519
Hydrolysis-Acidogenesis	Romboutsia	9.9449	6.1445	6.5358	6.1117
Hydrolysis-Acidogenesis	Clostridium	6.7972	3.2986	3.9553	3.9012
Hydrolysis-Acidogenesis	Candidatus_Caldatribacterium	5.6503	7.3843	8.3725	7.6163
Hydrolysis-Acidogenesis	Aneurinibacillus	4.0192	3.1736	0.0019	0.0008
Hydrolysis-Acidogenesis	Paraclostridium	3.3119	1.183	5.5053	6.3737
Hydrolysis-Acidogenesis	Intestinibacter	1.7513	1.001	1.2289	1.0923
Hydrolysis-Acidogenesis	Terrisporobacter	1.4547	0.8461	1.2954	1.4248
Hydrolysis-Acidogenesis	Proteiniphilum	1.4261	1.8129	1.4678	1.6665
Hydrolysis-Acidogenesis	unclassified_Anaerolineaceae	1.215	1.0294	0.9587	1.0365
Hydrolysis-Acidogenesis	Sedimentibacter	0.8454	0.9524	1.9163	1.8117
Hydrolysis-Acidogenesis	Tissierella	0.8288	0.3199	0.4342	0.2928
Hydrolysis-Acidogenesis	unclassified_Synergistaceae	0.6297	0.5868	0.2454	0.1787
Hydrolysis-Acidogenesis	Turicibacter	0.5643	0.7471	0.4018	0.4588
Hydrolysis-Acidogenesis	Bacillus	0.2296	0.3237	0.8781	0.2566
Acetogenesis	Syntrophomonas	0.9686	0.9025	0.8808	0.728
Acetogenesis	Propionicimonas	1.2552	0.6442	0.4891	0.4624
Acetogenesis	Petrimonas	0.8408	1.1228	0.7345	0.7116
Acetogenesis	Acetomicrobium	0.7196	0.7345	2.1958	1.617
Methanogenesis	Methanosarcina	19.2141	22.277	31.5987	33.9367

Methanogenesis	Methanothrix	1.6825	2.0413	0.0448	0.0444
Methanogenesis	Methanobacterium	0.5975	0.3069	2.4237	1.855
Bulk and foaming	Candidatus_Microthrix	0.3995	0.1587	0.9909	0.1934
AD not related	unclassified_D8A-2	0.7634	3.0751	5.5068	4.3716
AD not related	Advenella	0.3031	0.3567	1.7235	0.3342
AD not related	unclassified_Dojkabacteria	0.6771	1.3341	0.7556	1.5749
AD not related	unclassified_Methylococcaceae	1.8462	0.044	0.0269	0.0168
AD not related	unclassified_67-14	1.3436	1.2649	1.7031	1.2487
AD not related	Amaricoccus	1.2994	0.6411	1.5888	0.7139
AD not related	Gordonia	1.9638	1.7694	0.95	1.5583
AD not related	Mycobacterium	1.3615	0.5504	0.6762	0.5241
AD not related	Dechlorobacter	0.0802	0.0227	0.4794	0.0082
AD not related	unclassified_DTU014	0.1738	0.7671	0.4348	0.5868
AD not related	IMCC26207	0.56	0.8088	0.7602	0.6327
AD not related	unclassified_Gaiellales	0.7141	0.0871	0.5449	0.0838
AD not related	unclassified_Armatimonadota	0.4919	0.1475	0.201	0.1124

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